

Figure 1

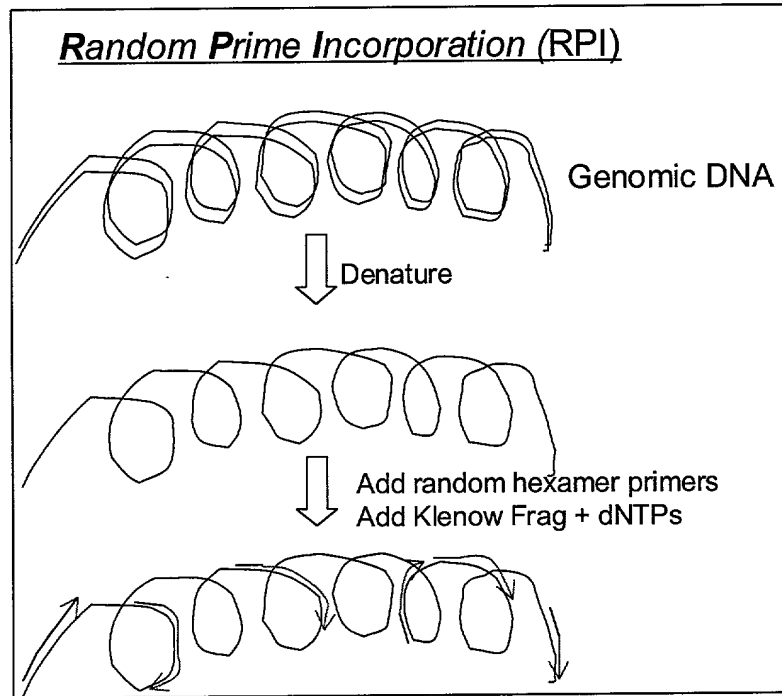


Figure 2

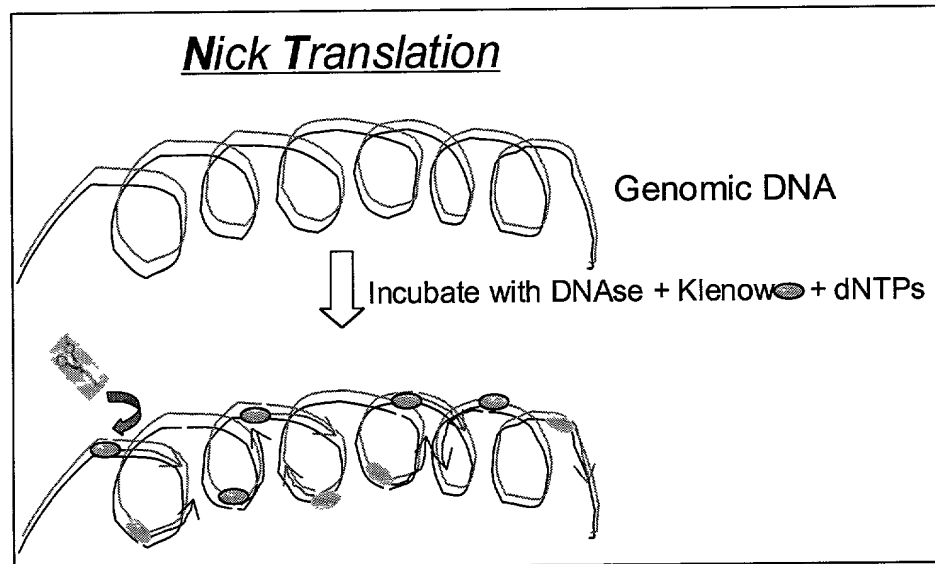


Figure 3

*Biased Primer Extension
Generated by DNase-Digested PCR Products*

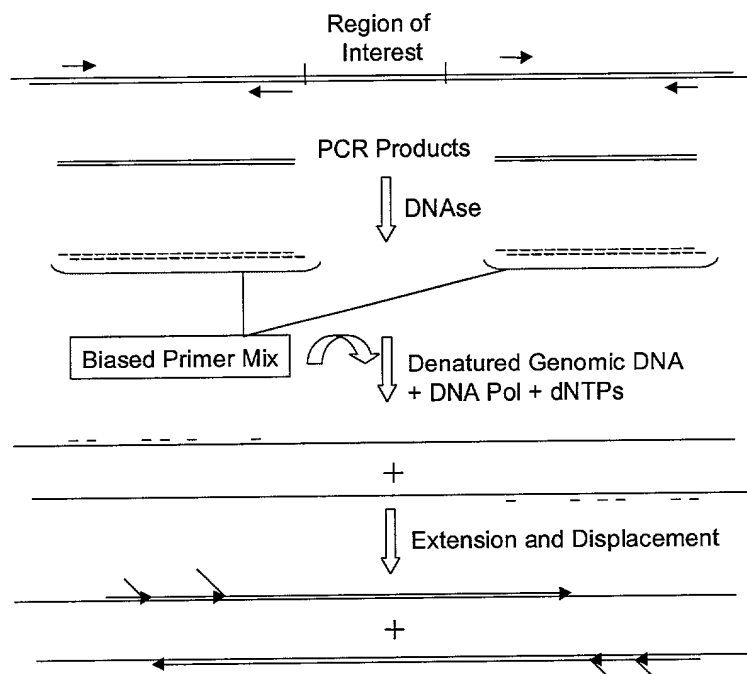


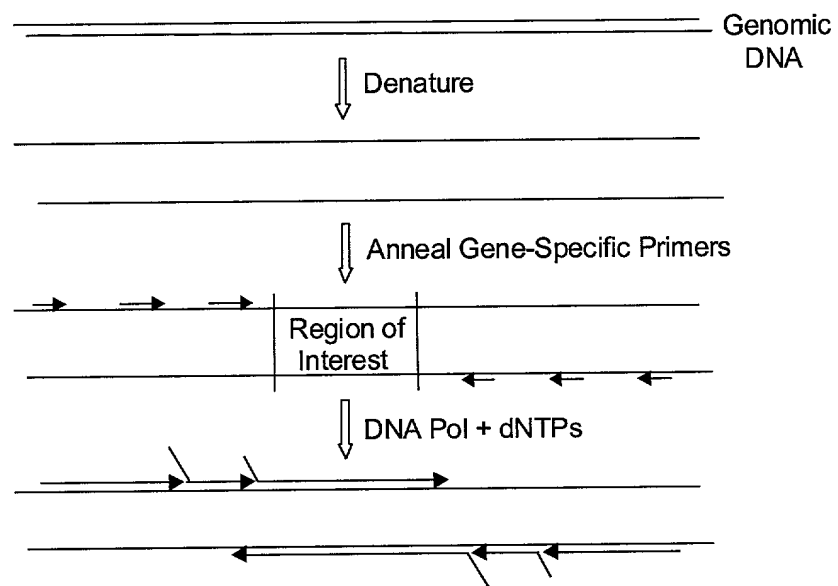
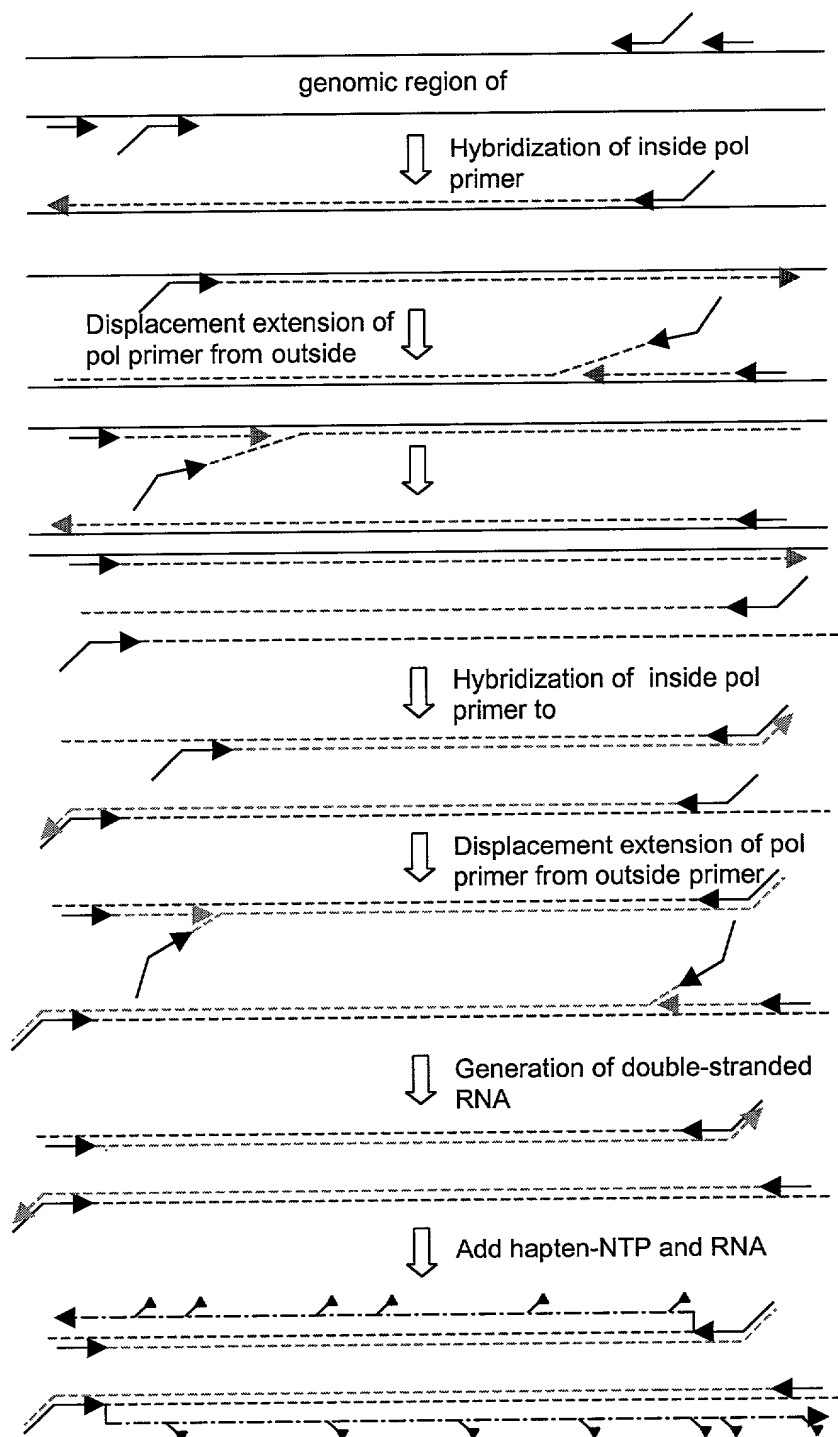
Figure 4*Gene-Specific Primer Extension using DNA Polymerase*

Figure 5

Extension Displacement Transcription Incorporation (EDTI)

00000722-0642001

Figure 6

1. 'A' Allele, CYP2D6*3, A2637 deletion, Frameshift resulting in zero enzyme activity

2637
 5'- GCTAACTGAGCACAGGATGACC -3' NH2 CYPwt(+)A2624,22mer,54%GC, Tm=63-64C
 5'- GCTAACTGAGCACAGGATGACC (A)30-3' NH2 CYPwt(+)A2624(A)30-3'NH2
 5'- CTAAGTGAAGCACAGGATGACC (A)30-3' NH2 CYPwt(+)A2625(A)30-3'NH2
 5'- CTAAGTGAAGCACAGGATGACC (A)30-3' NH2 CYPwt(+)A2625b(A)30-3'NH2
 5'- GCTAACTGAGCAC - GGATGACC -3' NH2 CYPmut(+)A2624,21mer,57%GC, Tm=61-63C
 5'- CTAAGTGAAGCAC - GGATGACC (A)30-3' NH2 CYPmut(+)A2624(A)30-3'NH2
 5'- CTAAGTGAAGCAC - GGATGACC (A)30-3' NH2 CYPmut(+)A2625(A)30-3'NH2
 5'- CTAAGTGAAGCAC - GGATGACC (A)30-3' NH2 CYPmut(+)A2625b(A)30-3'NH2
 5'- GCTGGATGAGCTGCTAACTGAGCACAGGATGACCCTGGGAGCCAGCCAGCC -3' Wild Type (+)
 5'- GCTGGATGAGCTGCTAACTGAGCAC - GGATGACCCTGGGAGCCAGCCAGCC -3' Mut (+)

2. 'B' Allele, CYP2D6*4, G1934A, Splicing defect resulting in zero enzyme activity

A. wt Probe - CYPwt(-)B1922 (C/A to mut at base 5) & CYPmut(+)B1922 (A/C to mut at base 13)

1934
 NH2 3'- GAGGGTGGGGGTCTCTGC -5' CYPwt(-)B1922,17mer,76%GC, Tm=66C
 5'- CTCCCACCCCCAGGACG -3' NH2 CYPwt(+)B1922- Target
 5'- CTCCCACCCCCAGGACG -3' NH2 CYPmut(+)B1922,17mer,71%GC, Tm=58-60C
 NH2 3'- GAGGGTGGGGGTCTCTGC -5' CYPmut(-)B1922- Target
 5'- CCCTTACCCGCATCTCCCACCCCCAGGACGCCCCCTTTCGCCCCCAACGGTCT -3' Wild Type (+)
 5'- CCCTTACCCGCATCTCCCACCCCCAGGACGCCCCCTTTCGCCCCCAACGGTCT -3' Mut (+)

B. CYPwt(-)B1930 (C/A to mut at base 13) and CYPmut(+)B1930 (A/C to wt at base 5)

NH2 3'- GGGTCTCTGCGGGGAAAG -5' CYPwt(-)B1930,17mer,71%GC, Tm=56C
 NH2 3'-(A)30 GGGTCTCTGCGGGGAAAG -5' CYPwt(+)B1930(A)30-3'NH2
 5'- CCCAAGACGCCCCCTTTC -3' NH2 CYPmut(+)B1930,17mer,65%GC, Tm=54C
 5'- CCCAAGACGCCCCCTTTC (A)30-3' NH2 CYPmut(+)B1930(A)30-3'NH2
 5'- CCCTTACCCGCATCTCCCACCCCCAGGACGCCCCCTTTCGCCCCCAACGGTCT -3' Wild Type (+)
 5'- CCCTTACCCGCATCTCCCACCCCCAGGACGCCCCCTTTCGCCCCCAACGGTCT -3' Mut (+)

3. 'C' Allele, CYP2D6*9, G2702-A2704 deletion, decreased enzyme activity

2702
 5'- GCAGAGATGGAGAAGGTGAGAG -3' NH2 CYPwt(+)C2691,22mer,55%GC, Tm=60C
 5'- GCAGAGATGGAGAAGGTGAGAG (A)30-3' NH2 CYPwt(+)C2691(A)30-3'NH2
 5'- CAGAGATGGAGAAGGTGAGAG (A)30-3' NH2 CYPwt(+)C2692(A)30-3'NH2
 5'- GCAGAGATGGA - - GGTGAGAGTG -3' NH2 CYPmut(+)C2691,21mer,57%GC, Tm=60C
 5'- GCAGAGATGGA - - GGTGAGAGTG (A)30-3' NH2 CYPmut(+)C2691(A)30-3'NH2
 5'- CAGAGATGGA - - GGTGAGAGTG (A)30-3' NH2 CYPmut(+)C2692(A)30-3'NH2
 3'- TGAAGTCCGGAAGGACCGTCTCTACCTCTTCCACTCTCACCAGACGGTGCCAC -5' Wild Type (-)
 3'- TGAAGTCCGGAAGGACCGTCTCTACCT - - CCACTCTCACCAGACGGTGCCAC -5' Mut (-)

4. 'E' Allele, CYP2D6*7, A3023C, H324P amino acid change results in zero enzyme activity

A. wt Probe - CYPwt(-)E3009 (T/C to mut at base 5) & CYPmut(+)E3009 (C/A to wt at base 15)

3023
 NH2 3'- CGAGTACTAGGATGTAGGC -5' CYPwt(-)E3009,19mer,53%GC,Pred Tm=57
 NH2 3'-(A)30 CGAGTACTAGGATGTAGGC -5' CYPwt(+)E3009(A)30-3'NH2
 5'- GCTCATGATCCTACCTCCG -3' NH2 CYPmut(+)E3009,19mer,58%GC,Pred Tm=59C
 5'- GCTCATGATCCTACCTCCG (A)30-3' NH2 CYPmut(+)E3009(A)30-3'NH2
 5'- TGGGGCCTCCTGCTCATGATCCTACATCCGGATGTGCAGC GTGAGCCCATC -3' Wild Type (+)
 5'- TGGGGCCTCCTGCTCATGATCCTACCTCCGGATGTGCAGC GTGAGCCCATC -3' Mut (+)
 -3038-Intron Start

B. CYPwt(-)E3018 (T/C to mut at base 14) and CYPmut(+)E3018 (C/T to wt at base 6)

NH2 3'- GGATGTAGGCCTACACGTC -5' CYPwt(-)E3018,19mer,58%GC, Tm=60
 5'- CCTACATCCGGATGTGCAG -3' CYPwt(+)E3018- Target
 5'- CCTACCTCCGGATGTGCAG -3' NH2 CYPmut(+)E3018,19mer,63%GC, Tm=62C
 3'- GGATGGAGGCCTACACGTC -5' CYPmut(-)E3018- Target
 5'- TGGGGCCTCCTGCTCATGATCCTACATCCGGATGTGCAGC GTGAGCCCATC -3' Wild Type (+)
 5'- TGGGGCCTCCTGCTCATGATCCTACCTCCGGATGTGCAGC GTGAGCCCATC -3' Mut (+)
 -3038-Intron Start

5. 'G' Allele, CYP2D6*8, G1846T, Stop codon, zero enzyme activity

1846
 5'- CACTCCGGTGGGTGATGG (A)30-3' NH2 CYPwt(+)G1840(A)30-3'NH2,18mer,67%GC, Tm=60
 NH2 3'-(A)30 GTGAGGCCACCCACTACC -5' CYPwt(-)G1840(A)30-3'NH2
 5'- CACTCCTGTGGGTGATGG (A)30-3' NH2 CYPmut(+)G1840(A)30-3'NH2,18mer,61%GC, Tm=57
 5'- GTGCCGCCTTCGCCACTCC GTGGGTGATGGGCAGAAAGGGCACAAAGCGGG -3'
 5'- GTGCCGCCTTCGCCACTCC GTGGGTGATGGGCAGAAAGGGCACAAAGCGGG -3'
 Exon 3 end -1846

6. T⁺ Allele, CYP2D6*6, T1795 deletion, Frameshift resulting in zero enzyme activity

5'- G C T G G A G C A G T G G G T G A C -3' NH2	CYPwt(+)-T1785, 18mer, 67%GC, Tm=59-61C
5'- G C T G G A G C A G T G G G T G A C (A)30-3' NH2	CYPwt(+)-T1785(A)30-3'NH2
5'- C T G G A G C A G T G G G T G A C (A)30-3' NH2	CYPwt(+)-T1786(A)30-3'NH2
5'- G C T G G A G C A G - G G G T G A C -3' NH2	CYPmut(+)-T1785, 17mer, 71%GC, Tm=58-60C
5'- G C T G G A G C A G - G G G T G A C (A)30-3' NH2	CYPmut(+)-T1785(A)30-3'NH2
5'- C T G G A G C A G - G G G T G A C (A)30-3' NH2	CYPmut(+)-T1786(A)30-3'NH2
5'- G G G C A A G A A G T C G C T G G A G C A G T G G G T G A C C G A G G A G G C C G C C T G C C T -3' Wild Type (+)	
5'- G G G C A A G A A G T C G C T G G A G C A G - G G G T G A C C G A G G A G G C C G C C T G C C T -3' Mut (+)	

7. 2D6/2D7/2D8 Controls - The 2D6/7/8 probes were designed in the 1600 region of the 2D6 gene. The purpose of the designs was to find region somewhere between the PCR primers where it would be easy to discriminate between 2D6 and its two pseudogenes, 2D7 and 2D8. The purpose of the designs is to demonstrate that the PCR amplicon is from the 2D6 gene, not one of the pseudogenes.

5'- G A C C A G G G G G A G C - A T A G G (A)30-3' NH2	CYP2D6wt(+)-1607(A)30-3'NH2
5'- G A C C T T G T G G A G C G C C A G (A)30-3' NH2	CYP2D7wt(+)-1607(A)30-3'NH2
5'- G A C C A G G A A A A G C - A C A G G (A)30-3' NH2	CYP2D8wt(+)-1607(A)30-3'NH2
1603- 5'- G A C C A G G A A A A G C - A C A G G G (A)30-3' NH2	CYP2D8wt(+)-1607b(A)30-3'NH2
5'- G G G A G A C C A G G G G G A G C - A T A G G G T T G G A G T G G G T G G T -3' 2D6 (+)	
5'- G G G A G A C C T T G T G G A G C G C C A G G G T T G G A G T G G G T G G C -3' 2D7 (+)	
5'- G G G A G A C C A G G A A A A G C - A C A G G G T T G G A G T G G G C G G C -3' 2D8 (+)	

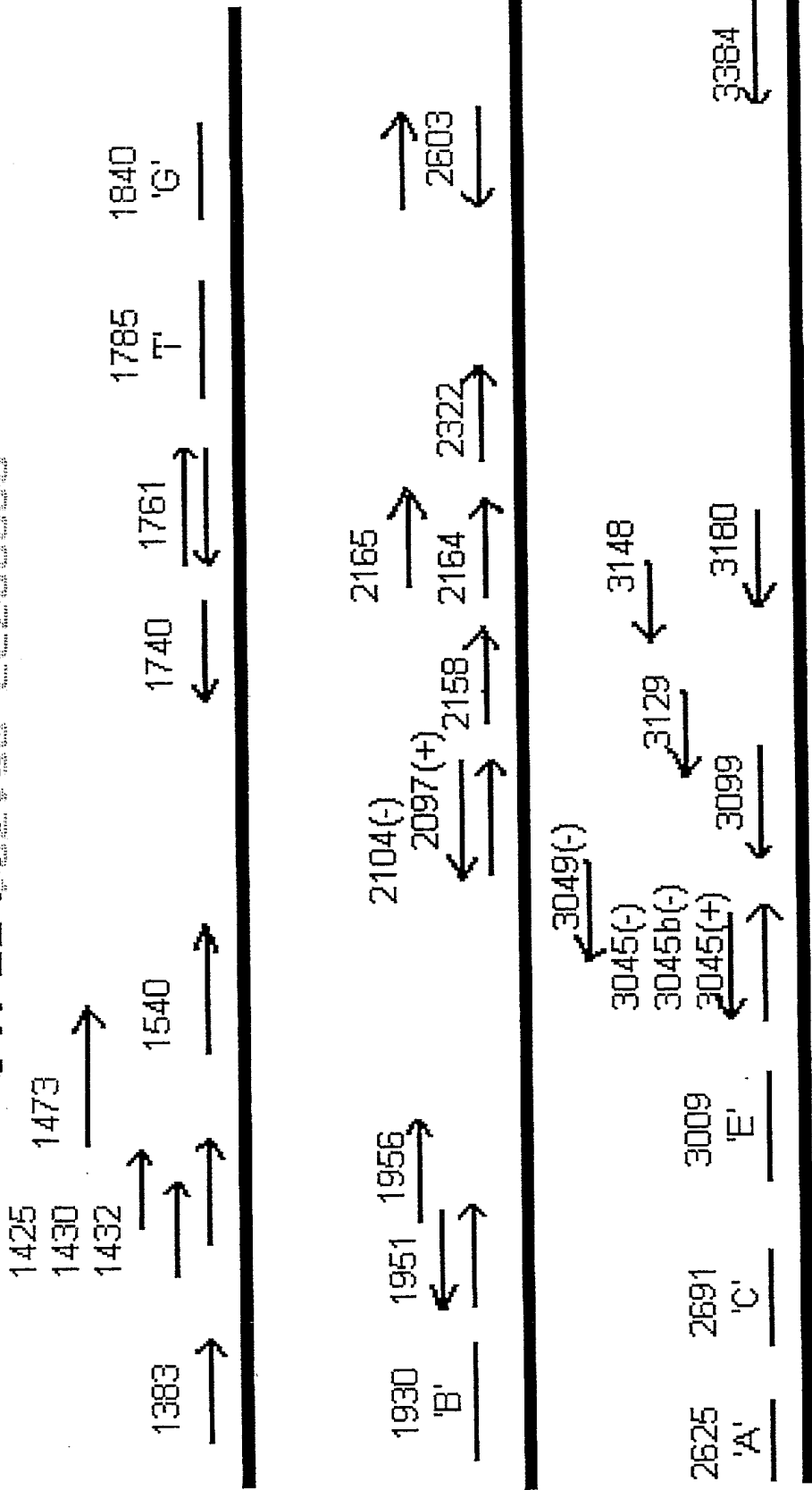
8. Pos/Neg Control probes- These probes were designed as true positive and negative control probes. They consist of the same semi-random sequence, with the positive control probe having a 5' Biotin.

5' Biotin- A T C A T T C C A A T C A T C C A T A T C A T C (A)25-3' NH2	CYP(+)-ran(A)25-5'Biotin, 3'NH2
5'- A T C A T T C C A A T C A T C C A T A T C A T C (A)25-3' NH2	CYP(+)-ran(A)25-3'NH2

1603- | 5'- G A C C A G G A A A A G C - A C A G G G (A)30-3' NH2

Figure 7

CYP2D6 Probes/Primers



CYPwt(+1383, CYPwt(+2097, CYPwt(-2104, and CYPwt(-3180 are published primer sequences.

1. Chen et al., Clinical Pharmacology and Therapeutics, Vol 60, 5:522-34

2. Heim M, Meyer UA. Lancet 1990; 336:529-32

CYPwt(+1540 and CYPwt(-3099 are primers obtained from Intek, referred to as MP3 and MP4 respectively.